NMR Investigations of the Interaction Between the Azo-dye Sunset Yellow and Fluorophenol

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Abstract

The interaction of small molecules with larger non-covalent assemblies is important across a wide range of disciplines. Here, we apply two complementary NMR spectroscopic methods to investigate the interaction of various fluorophenol isomers with sunset yellow. This latter molecule is known to form non-covalent aggregates in isotropic solution, and form liquid crystals at high concentrations. We utilise the unique fluorine-19 nucleus of the fluorophenol as a reporter of the interactions via changes in both the observed chemical shift and diffusion coefficients. The data are interpreted in terms of the indefinite self-association model and simple modifications for the incorporation of a second species into an assembly. A change in association mode is tentatively assigned whereby the fluorophenol binds end-on with the sunset yellow aggregates at low concentration and inserts into the stacks at higher concentrations.

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Introduction

The non-covalent self-association of molecules in solution is an important phenomenon across a range of areas in the chemical and biochemical sciences, from liquid crystal science and nanoscale engineering, to the formation of pathogenic protein assemblies.[1,2] Investigating and understanding the driving forces responsible for these assembly processes is therefore extremely important as this may provide information which enables the aggregation to be controlled[3,4] or prevented.[5-7] The interactions of small molecules with aggregates and assemblies is also an area of considerable interest, with applications ranging from hydrogen storage in metal organic frameworks[8] to the various assays used for monitoring the growth of amyloid fibrils.[9] In this latter case, the formation and assembly of protein fibrils is typically monitored using the dye thioflavin T in a fluorescence-based assay.[2] Binding of the dye to mature fibrils results in an increase in the fluorescence quantum yield and hence an increase in the recorded signal. While this assay is widely used within the fibril research community, the nature of the interaction between the dye and fibril is still the subject of much debate.[9,10] Small molecule-aggregate interactions has also found use in the resolution of pairs of enantiomers in NMR spectroscopy.[11-13] Chiral liquid crystal phases, in which the liquid crystal director aligns in the strong static magnetic field,[13] are added to a sample, resulting in different strength interactions for each enantiomer, enabling their resolution in the NMR spectrum. While this application is not (yet) considered routine in NMR spectroscopy, it does demonstrate the potential utility of exploiting small molecule-aggregate interactions, and a need to understand the origin and mechanisms of these interactions.

NMR spectroscopy is (almost) uniquely placed as an analytical tool, being able to offer an atomic level of detail on molecular interactions via the modulation of easily measurable parameters such as chemical shift,[14] as well as being able to access bulk physical parameters characterising the sample such as diffusion coefficients and microscopic sample mobility.[15,16]

The aggregation behaviour of sunset yellow FCF (SSY, sodium (*E*)-6-hydroxy-5-((4-sulfonatophenyl) diazenyl) naphthalene-2-sulfonate) in both isotropic solution and its liquid crystal phases has been well studied using a variety of techniques, including x-ray,[17-19] optical scattering[18] and NMR spectroscopy.[20-22] The association is widely thought to be head-to-tail stacking of the monomer units with a slight twist, typical of H-type aggregation.[23] It therefore presents an ideal system for investigating the interaction of non-covalent assemblies and small reporter molecules as a function of the concentration of the aggregating species, and hence aggregate size. The small molecule probes used were chosen to share some structural characteristics with sunset yellow and possess a unique magnetically-active reporter nucleus not present in the sunset yellow. The three structural isomers of fluorophenol (*n*FP) therefore presented as ideal candidates. The phenol moiety of *n*FP closely mimics the sulfated phenyl group of SSY and the unique magnetic reporter is provided by the ^{19}F, which is 100% abundant, spin-1/2, with excellent receptivity, being 0.941 of that for ^{1}H.[24] The structures of sunset yellow and the flurophenols, with the atom numbering used, are shown in Figure 1. Based on previous studies of SSY aggregation using optical[18] and NMR spectroscopies,[22] addition of the *n*FP probe at a concentration of 1 mol% should result in typically no more than around one to two probe molecules per aggregate in

the solution, hence both probe-probe interactions and any potential disruption of the sunset yellow aggregates should be expected to be minimal.

In this paper we describe an NMR-based investigation of the interaction between assemblies of the well studied azo-dye sunset yellow FCF,[17,18,20-22] in its isotropic phase, and a small "probe" molecule fluorophenol, present at low relative concentration. Changes in the observed diffusion coefficients and chemical shifts were monitored as a function of sample composition for both the probe molecules and sunset yellow aggregates. The results are interpreted in terms of the non-disruptive interaction between the probe and the sunset yellow assemblies, assuming that there is fast exchange both within the sunset yellow aggregates and with the fluorophenol interaction. The chemical shift changes are modelled using a simple indefinite association model,[14] modified for the incorporation of a second molecule into the assemblies.

Materials and Methods

Sample Preparation

All chemicals were purchased from Sigma Aldrich (Dorset, UK), with the exception of deuterium oxide which was obtained from either Goss Scientific (Cheshire, UK) or Sigma Aldrich. Sunset Yellow FCF was purified by two rounds of ethanol precipitation prior to use.[17,18] All other chemicals were used as obtained. A stock solution of 961 mM sunset yellow was prepared with the concentration determined by UV/vis spectrophotometry using a molar extinction coefficient of 8270 M^{-1} cm^{-1} at 523 nm, determined using samples of various concentrations at multiple pathlengths. This stock solution was then split into aliquots, into some of which were added

various isomeric monofluorophenol probes at a concentration of 1 mol%. These solutions were then diluted appropriately to obtain a series of samples with the desired final concentrations of sunset yellow both with, and without, the small molecule probes being present. At all concentrations the samples were confirmed to be in the isotropic phase by the observation of a singlet in the deuterium (^{2}H) NMR spectrum. The presence of any mesophase would have resulted in a quadrupole splitting of the D_2O solvent resonance due to the anisotropic nature of the sample.[20]

NMR Spectroscopy

All NMR data were acquired using a Varian VNMRS 600 spectrometer (Yarnton, UK) equipped with a X{^{1}H-^{19}F} broadband probe including an actively shielded *z*-gradient capable of 0.7 T m^{-1}. The sample temperature was maintained at 298 K for all experiments. Deuterium spectra (^{2}H) were obtained using the field-frequency lock channel. NMR data were processed using either Mnova NMR (Santiago de Compostela, Spain) or DOSY Toolbox[25] as appropriate. Diffusion ordered spectra were obtained using the Oneshot sequence[26] with a typical diffusion labelling period Δ of 100 ms in duration. 16 or 32 pulsed field gradient points were used with intensities between 0.0452 and 0.5650 T m^{-1}, equally spaced in g^2. The gradient durations used were typically between 1.5 and 3 ms. The stimulated echo attenuation data were fitted to the Stejskal-Tanner equation:

$$s(g) = s(0)\exp\left(-\gamma^2 g^2 \delta^2 D\Delta'\right) \quad (1)$$

where γ is the magnetogyric ratio of the diffusing nucleus, g and δ describe the strength and duration of the applied magnetic field gradient, and Δ' is the diffusion labelling period suitably modified for the Oneshot sequence.[26]

Chemical Shift Variation Modelling

The concentration dependent ^{1}H chemical shifts of sunset yellow were modelled using the isodesmic model[14] which, for clarity, will be described here briefly. In the fast exchange limit, the observed chemical shift can be written as the weighted average of the chemical shifts for the free monomer δ_{mon}, the molecules at the ends δ_{end} and those in the interior of the aggregates δ_{int}:

$$\delta_{obs} = \alpha\delta_{mon} + \lambda\delta_{end} + \xi\delta_{int} \quad (2)$$

where α, λ and ξ are the mole fractions of the free monomer and molecules at the ends and in the interior of the stacks respectively. These mole fractions by necessity sum to unity. This approach considers only nearest-neighbour contributions to the change in chemical shift. The inclusion of next-nearest-neighbour interactions has be shown to offer no significant improvement in modelling or understanding indefinite association.[14] It can be shown that the monomer mole fraction in solution at a given concentration, is:

$$\alpha = \frac{2K_{eq}c_T + 1 - \sqrt{4K_{eq}c_T + 1}}{2\left(K_{eq}c_T\right)^2} \quad (3)$$

where c_T is the total concentration of the solution and assuming that the equilibrium constants K_{eq} are equal for each subsequent addition of a monomer to the growing aggregate. Similarly, the mole fractions λ and ξ can be derived:[14]

$$\lambda = \frac{2\alpha^2 K_{eq}c_T}{1 - \alpha K_{eq}c_T} \quad (4)$$

$$\xi = \frac{\alpha^3 K_{eq}^2 c_T^2}{\left(1 - \alpha K_{eq}c_T\right)^2}$$

Combining eq 2, 3 and 4, with the additional simplifying assumption that the chemical shift of a molecule at the end of the stack is just the average of that for the

free monomer and a molecule in the interior, i.e. $\delta_{end} = (\delta_{mon} + \delta_{int}) / 2$, leads to the following model for the observed chemical shift after algebraic manipulation:[14]

$$\delta_{obs} = \delta_{mon} + \left(\delta_{int} - \delta_{mon}\right)\frac{2K_{eq}c_T + 1 - \sqrt{4K_{eq}c_T + 1}}{2K_{eq}c_T} \tag{5}$$

This model can then be fitted to the concentration dependent changes in the observed chemical shift using standard least-squares methods,[27] with all proton environments fitted simultaneously.

The incorporation of a second molecule *B*, present at low concentration, into stacks of another *A*, is also treated by Martin.[14] In this case, two equilibria are considered: the association of a molecule of *B* with the end of the stack of *A*, denoted A_e; and the insertion of *B* into a growing stack of *A*, which can be considered to be equivalent to joining two ends together. These equilibria are given below:

$$\begin{aligned} B + A_e &\Leftrightarrow BA_e \quad K_1 = \frac{b}{a}\left[A_e\right] \\ BA_e + A_e &\Leftrightarrow A_eBA_e \quad K_2 = \frac{c}{b}\left[A_e\right] \end{aligned} \tag{6}$$

where *a*, *b* and *c* are the mole fractions of the probe molecule *B* in free solution, at the ends and within the stacks respectively. The concentration of the ends of the stacks of *A* can be shown to be:[14]

$$\left[A_e\right] = 2c_T\left(1 - \alpha K_{eq}c_T\right) \tag{7}$$

where K_{eq} is the equilibrium constant for the aggregation of *A* and α is the mole fraction of *A* monomers in the solution (eq 3). The mole fractions *a*, *b* and *c* are given by:[14]

$$a = \frac{1}{1 + K_1[A_e] + K_1K_2[A_e]^2} \qquad (8)$$

$$b = \frac{K_1[A_e]}{1 + K_1[A_e] + K_1K_2[A_e]^2}$$

$$c = \frac{K_1K_2[A_e]^2}{1 + K_1[A_e] + K_1K_2[A_e]^2}$$

As for the case of the indefinite association of a single species, the observed chemical shift for the probe molecule *B*, in the presence of assemblies of *A*, is given by a similar expression to eq 2:

$$\delta^B_{\text{obs}} = a\delta^B_{\text{mon}} + b\delta^B_{\text{end}} + c\delta^B_{\text{int}} \qquad (9)$$

The underlying assumptions behind this approach are that the exchange of *B* is in the fast exchange limit, and that the presence of the second molecule does not perturb the stacks of *A*. Weller has presented a more sophisticated model for when the assumption of an excess of *A* no longer holds,[28] however, this approach is not needed in the analysis performed here. Data analysis was performed using the open source SciPy modules of the python programming language,[29]

Results and Discussion

NMR investigations of aggregating systems typically utilises concentration dependent changes in chemical shifts to reveal the underlying equilibria.[30,31] Bulk parameters, such as diffusion coefficients, have also been demonstrated to be useful.[22,32,33] In this study, a combination of both diffusion measurements and changes in the chemical shift of the ^{19}F moiety on the probe molecule are used to probe the interaction between of fluorophenol isomers with sunset yellow assemblies.

Diffusion Behaviour

The results of NMR diffusion measurements performed on a series of sunset yellow samples, with varying concentration, containing 1 mol% 3-fluorophenol as the small molecule probe, are shown in Figure 2. As reported previously, the diffusion coefficients observed for sunset yellow decrease with increasing concentration due to the formation of large assemblies in solution.[22] This trend is observed both in the presence and absence of the 3-fluorophenol, with the data obtained from the two series of samples overlaying extremely well (shown as the filled triangle and circles in Figure 2), indicating that the presence of the small molecule probe does not appear to affect the diffusion behaviour, and by inference, the aggregation state of the sunset yellow. The ^{1}H signals of the fluorophenol are difficult to observe in the presence of sunset yellow due to large differences in concentration and spectral crowding. Monitoring its diffusion properties via the fluorine substituent, however, allows the behaviour of the probe to be monitored without the interference of "background" signals arising from the sunset yellow. The observed diffusion coefficients of the ^{19}F probe with SSY are shown as the open circles in Figure 2. These follow a similar trend to that observed for SSY, that is, the diffusion coefficients decrease with increasing sunset yellow concentration. The obtained values for 3FP and SSY are, however, not the same. This suggests that the probe molecules, if they are associated with the SSY aggregates, spend at least some portion of their time disassociated in free solution. An alternative explanation is that there is no association between the fluorophenol and sunset yellow and that the trend in probe diffusion coefficient may be the result of increasing effective viscosity as the concentration of sunset yellow increases, or self-association of the fluorophenol molecules themselves. In order to determine whether this was the case, the diffusion coefficient of the probe was measured in the absence of sunset yellow, at the same relative concentration (i.e. 1

mol% of the SSY concentration). These results are plotted as a function of the corresponding sunset yellow concentration and shown as the filled squares in Figure 2. These data show that there is no significant detectable change in the diffusion coefficient over the concentration range studied, indicating that there is little self-association of the probe molecules at the concentrations of interest and hence this is not responsible for the variation in its diffusion coefficient when in the presence of sunset yellow.

In order to determine the influence of the larger microscopic viscosity at increasing sunset yellow concentrations, the diffusion coefficient of the residual HOD signal was also measured (see Figure S1 in Supplemental Information). This showed a linear decrease with increasing sunset yellow concentration, reflecting an effective increase in the solution microscopic viscosity. This variation in solvent diffusion coefficient was used to apply a "viscosity correction" to the diffusion coefficients for 3-fluorophenol on its own, as follows:

$$D_{3\mathrm{FP},free}^{corr} = \frac{D_{\mathrm{HOD}}}{D_{\mathrm{HOD}}^{0}} D_{3\mathrm{FP},free} \tag{10}$$

where D_{HOD} is the diffusion coefficient of the HOD signal at a given concentration of sunset yellow and $D_{\mathrm{HOD}}{}^{0}$ is the same extrapolated to infinite dilution. This viscosity-corrected diffusion data is shown as the open squares in Figure 2. These data show that while the diffusion coefficients for the ^{19}F probe-only samples do now decrease with increasing sunset yellow concentration, it does not do so in a manner which would account for the observed trend in the case of the probe molecule plus sunset yellow. The fact that the probe molecule's diffusion coefficient in the presence of SSY falls midway between that of each on its own suggests that, in the probe-sunset yellow system, the observed probe diffusion coefficient $D_{3\mathrm{FP}}$ represents an average of

the free probe in solution $D_{3FP,free}$ (viscosity corrected) and the sunset yellow aggregates interacting with the probe molecules D_{SSY}:[16]

$$D_{3FP} = \chi_{free} D^{corr}_{3FP,free} + \chi_{asc} D_{SSY} \quad (11)$$

with the requirement that $\chi_{free} + \chi_{asc} = 1$. This expression is valid in the case of fast exchange between the free and bound probes on the timescale of the diffusion labelling period Δ and therefore results in the observed monoexponential behaviour of the measured echo attenuation profiles.[16] Similar results were obtained for the two other structural isomers of fluorophenol (see Figure S2 in Supplemental Information).

The data presented in Figure 2 allows the degree of association between the probe molecules and the sunset yellow aggregates to be determined via rearrangement of eq 11:

$$\chi_{asc} = \frac{D_{3FP} - D^{corr}_{3FP,free}}{D_{SSY} - D^{corr}_{3FP,free}} \quad (12)$$

The results of this analysis, performed for each of the three structural isomers of fluorophenol, is shown in Figure 3. Two features are readily apparent from these data: firstly, the three probe molecule isomers show similar qualitative and quantitative trends in their interaction with the sunset yellow aggregates as viewed by the diffusion NMR data, suggesting that the position of the fluorine moiety has little effect on the interaction between the fluorophenol and sunset yellow; and secondly, the interaction behaviour shows two distinct regions as a function of SSY concentration with the changeover occurring at approximately 70 mM. A similar observation has been made previously in a study of the aggregation properties of various chlorhexidine salts in aqueous solution.[34] This two-region behaviour of the

associated probe-SSY mole fraction as a function of concentration was modelled as a biexponential function of SSY concentration using:

$$\chi_{\text{asc}} = a_1 \exp\left(-\frac{c_T}{b_1}\right) + a_2 \exp\left(-\frac{c_T}{b_2}\right) + a_0 \quad (13)$$

where c_T is the concentration of sunset yellow. The parameters obtained from these fits are given in Table 1 and the relative percentage amplitudes of the two components in Table 2. Interestingly there is an apparent correlation between the b_2 parameter describing the second exponential component and the pK_a of the fluorophenol probes.[35] The b_2 parameter increases in magnitude as the fluorophenol decreases in acidity. The change over in dominant exponential component also appears to correlate with the fluorophenol probe pK_a. There is no such correlation apparent for the b_1 parameter, with it being similar for both 2FP and 3FP, although, there is an increase of a factor of ~3 for the 4-fluorophenol isomer. The differences in these parameters may be related to the hydrogen bonding ability of the different isomers,[36,37] which, in addition to the π-π stacking interaction,[38] is presumed to be important in the interaction between the fluorophenols and sunset yellow. Hydrogen bonding interactions have also been shown to play a role in the interaction between various fluorophenols and alkoxystilazole-based liquid crystal forming systems.[35]

Chemical Shift Changes

The variation of the ^{1}H chemical shifts of sunset yellow and other sulfonated azo-dyes as a function of sample concentration is well documented.[20,39] Typically, changes in chemical shift resulting from self association are described in terms of an isodesmic[14,30,40,41] or indefinite cooperative model.[14,30] For the samples studied here, similar trends to those reported in the literature[20,21] are observed for the concentration

dependence of the ^{1}H chemical shifts of sunset yellow both in the absence and presence of the three fluorophenol probes. At all concentrations, the observation of a single set of resonances is consistent with the monomer-aggregate equilibrium being in the fast exchange regime on the NMR timescale.[20-22] There is a general monotonic decrease in the observed ^{1}H chemical shift as a function of increasing concentration as shown in Figure 4(a). This results from an increase in shielding afforded by the π-π aromatic interaction stacking upon self association.[20] Upon addition of the fluorophenol probes there is little observable effect on the absolute values of the sunset yellow ^{1}H chemical shifts or on their change as a function of concentration. As an example, the data for SSY plus 3-fluorophenol is shown in Figure 4(b). These chemical shift data can be analysed in terms of the isodesmic model given in eq 5, resulting in the equilibrium constants presented in Table 3. The equilibrium constants are broadly similar, however, as with the diffusion data presented above, there is a correlation between the equilibrium constants returned and the pK_a of the fluorophenol.[35] These values suggest that the presence of the fluorophenol leads to a slight increase in the stability of the sunset yellow assemblies, presumably involving hydrogen bonding in addition to the π-π interactions. More recently,[21] it has been suggested that the isodesmic model is not necessarily a good description of the assembly process in sunset yellow, implying the underlying assumption of a single equilibrium constant for each microscopic step is not valid, i.e. the addition of the next monomer unit to the aggregate is not independent of the number of monomers in the aggregate.[21]

The variation in the fluorine-19 chemical shift of the fluorophenol probes demonstrates a markedly different trend. Figure 5(a) shows the ^{19}F spectrum of the 3-

fluorophenol probe molecule, present at 1 mol%, over the range of sunset yellow concentrations. Initially, as the concentration of sunset yellow increases there is a change to more negative chemical shift. This is consistent with increased shielding of the ^{19}F nucleus as it interacts with the π-system of the sunset yellow aggregates,[20] most likely via π-π stacking interactions.[38] Once the sunset yellow concentration reaches around 100 mM, however, there is a change in the behaviour of the ^{19}F chemical shift. Above this concentration the chemical shift becomes more positive with increasing concentration, indicating that there is an increase in the deshielding contribution to the ^{19}F chemical shift. The observed chemical shifts for each of the three probe molecules are plotted in Figure 5(b). In all cases similar trends are seen, both in terms of the magnitude of the observed changes in chemical shift as a function of concentration and the point at which the trend switches over from a shielding to deshielding. The only difference is the absolute value of the chemical shift, which is consistent with the substitution pattern around the phenol ring.[42] It is also important to note that, since fluoroaromatic chemical shifts are extremely sensitive to environment, particularly with regard to solvent dependence,[42] there was no observable change in the ^{19}F chemical shift in the absence of sunset yellow for any of the fluorophenol probes over the concentration range studied (see Figure S4 in Supplemental Information).

The isodesmic model used to analyse the concentration dependent changes in the ^{1}H chemical shifts of sunset yellow can be modified to account for the incorporation of a second molecular species into aggregate stacks.[14] This situation describes the proposed association of the fluorophenol probes with sunset yellow. The results of applying this modified isodesmic model, as described in eq 9, to the observed changes

in ^{19}F chemical shift are shown as the solid lines in Figure 5(b). The modified isodesmic model comprises two equilibria: one for the association of a probe molecule with the end of a sunset yellow stack; and a second for the incorporation of a fluorophenol within the stack itself. This latter process is equivalent to the joining of two ends of two stacks together via a probe molecule. The equilibrium constants obtained from these fits are given in Table 4. These results suggest that at low concentrations, when the stacks of sunset yellow are generally short, i.e. less than around 100 monomer units,[22] the predominant interaction of the fluorophenol is with the ends of the sunset yellow stack. This results in an increase in shielding due to ring current effects, and hence a change to more negative chemical shifts. The relative proportion of stack-ends is inversely related to the length of the stack. Above the change over concentration, around 100 mM, where the ^{19}F chemical shift changes to more positive values, the sunset yellow stacks are generally long, comprising hundreds of molecules.[22] Under these conditions, the relative concentration of stack ends is small, and hence the fluorophenol probe molecules are more likely to be found incorporated into the stacks. The deshielding effect observed here would suggest that the fluorine group is typically found on the interior of the stacks and hence deshielded via ring current effects.[43]

In order to gain further insight into the nature of the interaction between the fluorophenol probe and sunset yellow, ^{1}H-^{1}H NOESY and ^{19}F-^{1}H HOESY[44] (Heteronuclear Overhauser Effect SpectroscopY) experiments were attempted. The aim was to look for through space correlations which would shed light on the relative orientation of the fluorophenol molecule when inserted into, or bound to the end of, a sunset yellow aggregate. Unfortunately, as with similar experiments attempted solely

on sunset yellow,[22] these experiments were not successful, principally due to the low concentration of the fluorophenol probe resulting in low sensitivity in the ^{19}F-^{1}H HOESY experiments. Increasing the concentration of fluorophenol would risk disrupting the sunset yellow aggregation and hence perturbing the processes under investigation. The degree to which disruption occurs is currently under investigation.

There are a number of reports in the literature describing aromatic stacking interactions between molecules with similar structures such as pyrimidines and purines,[45,46] purine and indoles,[47] and caffeine and adenosine.[28] Several of these studies employ more sophisticated versions of the isodesmic model,[28] incorporating *AB*-type interactions in addition to *AA*- and *BB*-type. Weller also develops a model where the aggregating molecule, i.e. species *A*, is no longer in excess and hence the modified isodesmic model used here cannot be applied.[28] These systems are in general investigated via changes in the ^{1}H chemical shifts, and do not show the same biphasic trends as seen with the ^{19}F chemical shifts presented here. This is likely due to the investigated pairs of molecules being more similar, differing only by the placement of methyl or hydroxyl groups, and therefore they present similar modes of interaction. In the case of the system investigated here, i.e. fluorophenol and sunset yellow, the monomer units and probe molecules have greater differences in size and gross functionality, resulting in the possibility that fluorophenol undertakes a different binding mode compared to the addition of another sunset yellow monomer to the stack.

Conclusions

The interaction of small molecules with self-assembling or aggregating systems is important across a wide range of research areas. In this article we have investigated the interaction of three isomers of fluorophenol with sunset yellow, which is known to form aggregates of tens to hundreds of molecules while in isotropic solution. The addition of fluorophenol at a low relative concentration of 1 mol%, results in extremely limited disruption of the sunset yellow aggregation as judged from the concentration dependence of both the diffusion coefficients and ^{1}H chemical shifts observed for sunset yellow. The ^{19}F-detected diffusion measurements for the fluorophenol probe molecules show that its addition results in a fast exchange process between interacting with the aggregates and being in free solution, rather than total incorporation into the sunset yellow aggregates. The observed ^{19}F chemical shifts show a biphasic trend, with its change over point at a similar concentration to the changes in the associated mole fraction derived from the diffusion measurements. The shielding-deshielding trend in ^{19}F chemical shift is distinct from previously reported chemical shift trends for the association of pairs of similarly structured molecules, however, simple modification of the isodesmic model can be used to account for it. The different response of the ^{19}F chemical shifts contrasted with ^{1}H may be the result of differences in the nature of the π-π interaction as a result of the fluorine substituent and differences in the molecular electric quadrupole moments.[48-50] Unfortunately, due to the low concentrations involved and numerous exchange processes occurring in this system, Overhauser effect experiments performed with the aim of gaining further insight into the nature of the fluorophenol-SSY interaction were unsuccessful. The interaction of similar probe molecules with sunset yellow containing other NMR-active nuclei, such as phosphorous-31, are currently underway.

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Supporting Information Available: Data for the HOD diffusion measurements and corresponding diffusion data for 2- and 4-fluorophenol structural isomers, along with ^{19}F NMR data. This information is available free of charge via the Internet at http://pubs.asc.org.

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Tables

Table 1: Parameters extracted from the fitting of eq 13 to the experimental data presented in Figure 3.

Sample	a_1	b_1 (M)	a_2	b_2 (M)	a_0
2FP	-0.899	0.019	-0.713	0.877	0.909
3FP	-0.639	0.017	-0.917	1.096	1.09
4FP	-0.283	0.059	-0.852	1.202	1.05

Table 2: Percentage amplitudes for the two components of eq 13 fitted to the data in Figure 2.

Sample	a_1 component	a_2 component
2FP	53%	47%
3FP	46%	54%
4FP	41%	59%

Table 3: Equilibrium constants for the association of sunset yellow obtained from the concentration dependence of the ^{1}H chemical shifts using the isodesmic model (eq 3).[14]

Sample	K_{eq} (M)
SSY only	6.7 ± 0.3
SSY + 1% 2FP	8.5 ± 0.5
SSY + 1% 3FP	7.6 ± 0.3
SSY + 1% 4FP	6.8 ± 0.3

Table 4: Equilibrium constants for the association of fluorophenol and sunset yellow obtained from the concentration dependence of the ^{19}F chemical shifts using the modified isodesmic model (eq 9).[14]

Sample	K_1 (M)	K_2 (M)

SSY + 1% 2FP	0.5 ± 0.7	5.2 ± 57
SSY + 1% 3FP	0.1 ± 0.1	1.3 ± 3.8
SSY + 1% 4FP	0.6 ± 0.4	1.3 ± 9.8

Figure Captions

Figure 1: Structure of the hydrazone tautomer **1** of sunset yellow, with the three isomers of fluorophenol used.

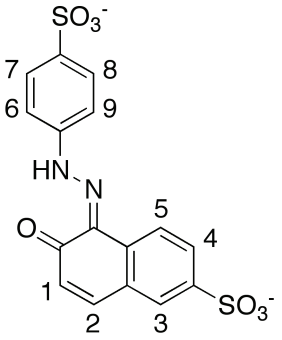
Figure 2: Diffusion coefficients for sunset yellow in the absence and presence of 3-fluorophenol, measured from ^{1}H for sunset yellow and ^{19}F for 3-fluorophenol. Note, the filled triangle and circle symbols overlay extremely well. The data for the 3-fluorophenol only sample is plotted at the corresponding sunset yellow concentration if it were to be present, i.e. the true concentrations are 1% of that on the abscissa.

Figure 3: Associated mole fractions χ_{asc} calculated from the diffusion coefficient data for each of the three isomers of fluorophenol. The lines indicate the best fits to eq 13.

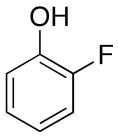
Figure 4: Plots of the observed ^{1}H chemical shift as a function of sunset yellow concentration in the (a) absence and (b) presence of 1 mol% 3-fluorophenol. The solid lines are the result of a global fit to the isodesmic model given in eq 5.

Figure 5: (a) shows the ^{19}F chemical shifts for 3-fluorophenol present in solutions of sunset yellow at a concentration of 1 mol%. The concentration values given on the figure are those for the sunset yellow. The spectra were acquired with equal numbers of transients. (b) shows the concentration dependence of the ^{19}F chemical shift for

each of the three fluorophenol isomers. The solid lines are derived from fitting the isodesmic model using eq 9.[14]



1



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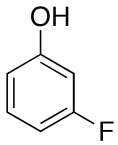

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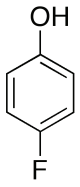

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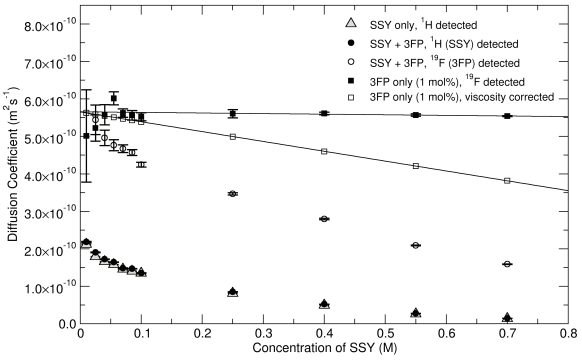
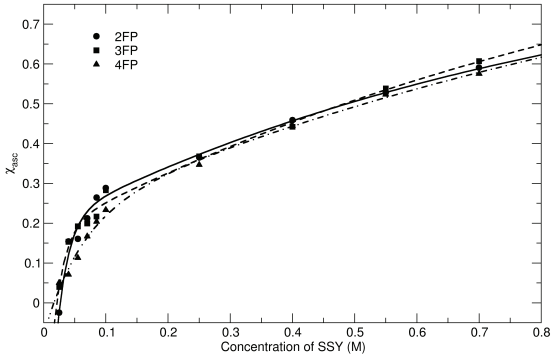
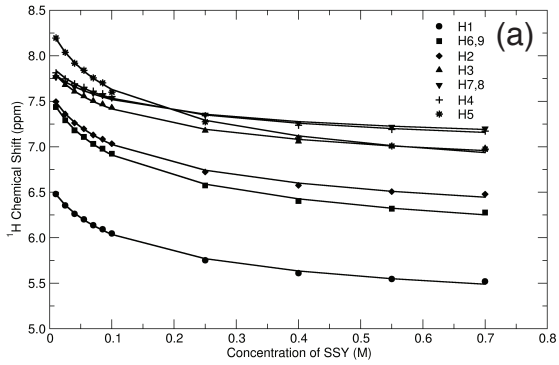
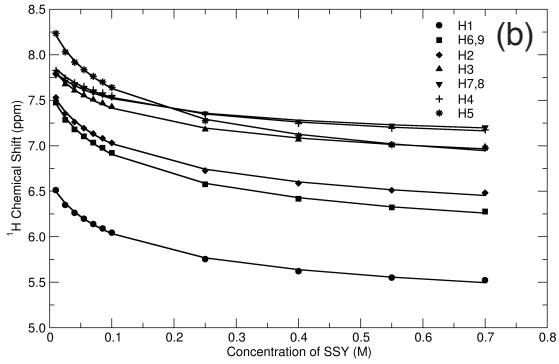
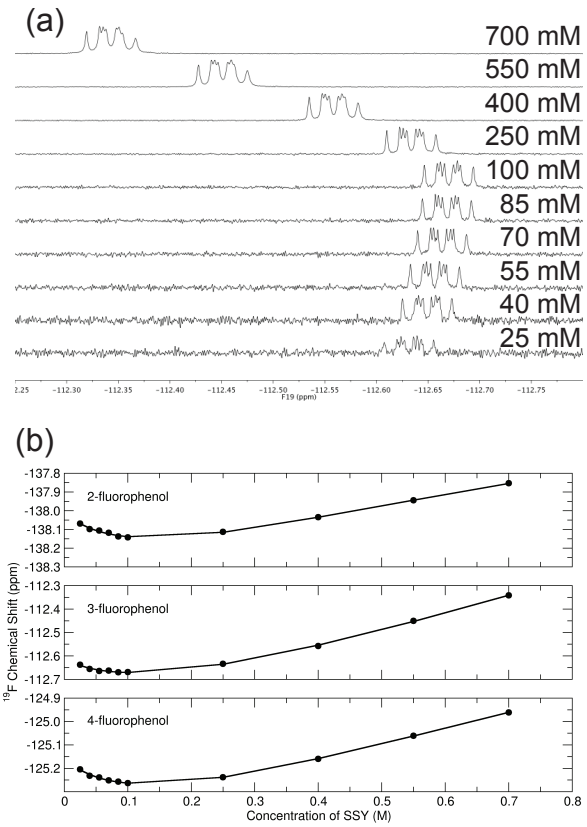

SSY only, ^{1}H detected
SSY + 3FP, ^{1}H (SSY) detected
SSY + 3FP, ^{19}F (3FP) detected
3FP only (1 mol%), ^{19}F detected
3FP only (1 mol%), viscosity corrected
Diffusion Coefficient ($m^2 s^{-1}$)
Concentration of SSY (M)
8.0×10^{-10}
7.0×10^{-10}
6.0×10^{-10}
5.0×10^{-10}
4.0×10^{-10}
3.0×10^{-10}
2.0×10^{-10}
1.0×10^{-10}
0.0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8


2FP
3FP
4FP
χ_{asc}
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Concentration of SSY (M)


(a)
H1
H6,9
H2
H3
H7,8
H4
H5
^{1}H Chemical Shift (ppm)
Concentration of SSY (M)


(b)
H1
H6,9
H2
H3
H7,8
H4
H5
^{1}H Chemical Shift (ppm)
Concentration of SSY (M)


(a)
700 mM
550 mM
400 mM
250 mM
100 mM
85 mM
70 mM
55 mM
40 mM
25 mM
-112.25
-112.30
-112.35
-112.40
-112.45
-112.50
-112.55
-112.60
-112.65
-112.70
-112.75
f19 (ppm)
(b)
2-fluorophenol
3-fluorophenol
4-fluorophenol
^{19}F Chemical Shift (ppm)
Concentration of SSY (M)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8